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REVIEW

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Expression, transcriptional regulation and functional analysis of phosphate transporter genes in plants

Abstract Phosphorus is one of the essential mineral nutrients required by all living cells. Phosphate mobilization into the plant is a complex process in which the absorption and translocation of this major nutrient are determined largely by the phosphate (Pi) transporters. In this paper, the recent progress on the plant phosphate (Pi) transporter genes, such as the molecular characterizations, expression patterns in response to Pi status, other inorganic nutrients, and the other factors, expression regulations via arbuscular mycorrhizal (AM) symbiosis, mechanisms of transcriptional regulation, functional identification approaches, and the gene engineering perspectives on improvement of plant phosphorus nutrition, etc., have been reviewed. The purpose of this paper is to provide a theoretical basis for further elucidation of the molecular mechanism of Pi transportation mediated by Pi transporters and to promote the generation of elite crop germplasms with a significant improvement in phosphorus use efficiency in the future.

Keywords plant phosphate (Pi) transporter gene, expression, transcriptional regulation, functional analysis

1 Introduction

As one of the major but least available and essential nutrients, phosphate (Pi) in the environment shows a low availability in the rhizosphere, which leads to numerous adaptations required for the survival of plants (Muchhal and Raghothama, 1999). Two transportation systems of Pi in plants, comprised of high-affinity Pi transporters and low-affinity Pi transporters, have been identified and play important roles in regulating Pi uptake and its mobilization within the plants (Drew and Saker, 1984; Shimogawara and Usuda, 1995; Muchhal and Raghothama, 1999). Therefore, enhancement of Pi uptake in plants during phosphorus deficiency has become an effective way for the improvement of plant phosphorus nutrition. The molecular characterization, regulated-expression pattern in response to Pi and arbuscular mycorrhizal (AM) symbiosis, transcription regulation mechanism, and the functions of plant Pi transporter genes have been reviewed in this paper.

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2 Molecular characterization of plant Pi transporters

The Pi transporters within plants are integral membrane proteins. They are mostly distributed in the cytoplasm membrane, plastid envelope, and mitochondrion membrane at the cellular level (Daram et al., 1999; Nakamori et al., 2002; Versaw and Harrison, 2002). Generally, the plant Pi transporters classified into the same subgroup show a high similarity degree with each other at the nucleic acid sequence level and the amino acid sequence level. The polypeptides of plant Pi transporters generally contain 12 membrane-spanning domains separated into two groups of six by a large charged hydrophilic region (Vance et al., 2003). At the nucleic acid level, the open reading frames of plant phosphate transporter generally vary from 1500 to 1800 nt, encoding 500–600 of amino acids. Genome sequence data indicate that plant genomes contain large numbers of genes predicted to encode Pi transporters, existing as a gene family (Okumura et al., 1998; Liu et al., 2008). In the model plant *Arabidopsis*, there have been over 10 Pi transporter genes identified thus far (Stefanovic

et al., 2007). In rice, nearly 13 phosphate transporter genes have been isolated (Ai et al., 2008). In the past several years, more genes encoding phosphate transporters have been also isolated from a number of other plant species, such as tomato (Liu et al., 1998a; Xu et al., 2007), barley (Rae et al., 2003; Schünmann et al., 2004a), maize (Nagy et al., 2006), *Medicago truncatula* (Nagy et al., 2005; Grunwald et al., 2009), duckweed (Hase et al., 2004), potato (Rausch et al., 2001; Gordon-Weeks et al., 2003), birch (Kiiskinen et al., 1997), *Lotus japonicus* (Nakamori et al., 2002), wheat (Zeng et al., 2002; Tittarelli et al., 2007; Miao et al., 2009), *Dunaliella viridis* (Li et al., 2006), and maritime pine (Tatry et al., 2009).

The important roles of plant Pi transporters are shown to be involved in the Pi acquisition from the soil/growth medium and the Pi translocation within the plants. Recently, other functions of plant Pi transporters have been explored. Huang et al. (2008) observed that a tree peony Pi transporter, PsMPT, also plays an important role in the release of ATP during bud dormancy. In *Arabidopsis*, overexpression of a high-affinity Pi transporter *PHT1;1* results in a slow rate of arsenic (As(V)) uptake in the transgenic plants, suggesting also that part of genes specifically involved in Pi uptake are transcriptionally regulated by As(V) (Catarecha et al., 2007). Another Pi transporter located in the thylakoid membrane, ANTR1, is also involved in several other physiologic roles, such as export of Pi produced during nucleotide metabolism in the thylakoid lumen back to the chloroplast stroma and balance of the trans-thylakoid H^+ electrochemical gradient storage (Pavón et al., 2008). The *Arabidopsis* Pi transporter PHT4;6 (At5g44370) regulates NaCl response in seedlings. Insertion mutations that disrupt the function of PHT4;6 cause NaCl hypersensitivity of *Arabidopsis* seedlings, which is characterized by reduced growth of the primary root, enhanced lateral branching, and swelling of root tips. It is also observed that the mutant phenotypes are exacerbated by sucrose, but not by equiosmolar concentrations of mannitol, and attenuated by low inorganic phosphate in the medium (Cubero et al., 2009). In the meantime, mutation of PHT4;6 causes phenotypic similarities with other mutants displaying root swelling, suggesting that PHT4;6 likely functions in protein N-glycosylation and cell wall biosynthesis (Cubero et al., 2009). Therefore, other functions of Pi transporter are involved in plants and the corresponding mechanisms are to be elucidated in the future.

Similar to those in *E. coli* and yeast, the functions of plant Pi transporters are mediated either by Pi:H^+ or Pi:Na^+ symport with different affinities for Pi (Tatry et al., 2009). Depending on the affinities for Pi, both high-affinity and low-affinity types have been identified (Smith et al., 2000). Generally, other than holding 12 transmembrane (TM) domains, the plant Pi transporters also contain two conserved motifs each consisting of eight and nine amino acids, located in the N- and C-terminal domains, respectively. Comparison analysis found that these two motifs are highly conserved among plant species, and even across all kingdoms, such as eubacteria, archea, fungi, and animals (Daram et al., 1999). The 12 transmembrane (TM) domains are generally interrupted by a large hydrophilic loop located between the transmembrane 6 and 7 (Vance et al., 2003), but the large hydrophilic loop is occasionally located between TM8 and TM9 (Daram et al., 1999).

3 Cellular location of the plant Pi transporters

Except for Pi acquisition from the soil and the Pi cellular translocation, Pi is also mobilized among the subcellular organs, such as the Pi being necessary to be exchanged among the cytoplasm, plastid and mitochondrion. Various approaches have been adopted and several phosphate transporters have been identified which are located in the chloroplast envelope, mitochondrion membrane and Golgi apparatus.

The Pi transporter PHT2;1 of *Arabidopsis*, sharing similarity with members of the Pi transporter family, which possess extended N termini and shares features with chloroplast transit peptides, is present in the chloroplast envelope by PHT2;1-green fluorescent protein (PHT2;1-GFP) fusion analysis (Versaw and Harrison, 2002). Based on Western blotting with two different peptide-specific antibodies analysis, Pavón et al. (2008) have demonstrated that Na^+-dependent inorganic phosphate (Pi) transporters in *Arabidopsis* ANTR1, homologous to the type I of mammalian putative anion transporter, is localized to the chloroplast thylakoid, which demonstrates the presumption that the presence of a specific, high-affinity Pi translocator is located in the spinach chloroplast envelope. The function of this Pi translocator is to take up Pi into the chloroplast from the cytosol to replenish the Pi pool in the stroma (Lunn and Douce, 1993). Except for the location of high-affinity phosphate transporters in plastids, a low-affinity orthophosphate (Pi) transporter also located in plastid, Pht2;1, has also been identified in potato, based on RNA gel blot analysis, reverse transcription-polymerase chain reaction (RT-PCR), promoter/GUS, and protein/green fluorescent protein (GFP) fusion studies (Rausch et al., 2004). These results imply that the translocation mechanism of Pi from the cytosol to chloroplast is complicated and involved phosphate transporters with different Pi-affinities.

Several phosphate transporters located in the mitochondrion membrane have been isolated in various plant species in the past decade. Based on DDRT-PCR (differential-display reverse-transcription polymerase chain reaction) and cDNA library screening methods, a 1.3 kb cDNA encoding a mitochondrial phosphate translocator protein (Pic) designated *Mpt1* (the first mitochondrial phosphate transporter reported in plant species), has

been isolated in birch. *Mpt1* contains an open reading frame of 1092 bases encoding a 364 amino acid polypeptide, much fewer than those in cytosol membrane and plastid envelope (Kiiskinen et al., 1997). Other mitochondrial phosphate transporter genes have also been identified in soybean, maize, rice, and *Arabidopsis* (Takabatake et al., 1999), *Lotus japonicus* (Nakamori et al., 2002), and tree peony (*Paeonia suffruticosa*) (Huang et al., 2008). It is currently known that most of the mitochondrial phosphate transporters in plants have generally about six of the conserved transmembrane domains, and nearly half of those targeted in cytosol membrane and plastid envelope.

The *Arabidopsis* PHT4;6 belong to the Major Facilitator Superfamily of permeases that share a significant sequence similarity to mammalian type-I Pi transporters (Cubero et al., 2009). Bioinformatic analysis and localization of PHT4-GFP fusion proteins indicate that five of the proteins are targeted to the plastid envelope, and the sixth resides in the Golgi apparatus (Guo et al., 2008). Therefore, the target of each member of the phosphate transporter in plants has a distinct location which is regulated by the polypeptide length, signal peptide characterizations at the N- and C-terminal, and the structure properties at the secondary or the three-dimensional level.

4 Expression patterns of plant Pi transporter genes

4.1 Tissue expression pattern

Plants assimilate phosphate (Pi) from the soil, which is largely determined by the capacity of plants to enhance Pi uptake during nutrient deficiency (McPharlin and Bieleski, 1987; Furihata et al., 1992). Over the past decade, it has been clearly found that the increase of Pi uptake in plants is correlated with an increased number of high-affinity Pi transporters assembled in the plasma membrane (Drew and Saker, 1984; Shimogawara and Usuda, 1995; Muchhal and Raghothama, 1999).The locations of transcripts and translated Pi transporters in plants are shown to be constitutive or organ and tissue specific. Most high-affinity Pi transporter genes are shown to be organ specific, with a pattern of being predominantly expressed in roots. It is noted that the earlier identified high-affinity Pi transporter genes *AtPT1* and *AtPT2* in *Arabidopsis* (Smith et al., 1997), and *MtPT1* and *MtPT2* in *M. truncatula* (Liu et al., 2008), are shown typically to have root-specific expression; no transcripts are detected in leaves. Conversely, the low-affinity Pi transporter genes, such as *Arabidopsis Pht2;1*, are predominantly expressed in green tissue, the amount of transcript staying constant in leaves irrespective of the Pi status of the shoot. However, there is a marginal increase of *Pht2;1* mRNA amounts in roots in response to Pi deprivation (Daram et al., 1999).

At the tissue level, different locations of Pi transporter transcripts or proteins were identified. Pi transporter gene *OjPT* and *StPT2* are expressed in root tip (Yu et al., 2001; Gordon-Weeks et al., 2003). Expressed in both leaves and roots, rice *OsLPT1* transcripts are detected mainly in mesophyll cells, xylem parenchyma and phloem cells in the leaves, and in the epidermis, exodermis, and vasculature surrounding metaxylem vessels in the roots (Ming et al., 2005). These results, combined with the data that this Pi transporter gene is predominantly expressed in roots under Pi-deficient condition, suggest that the OsLPT1 protein may be involved in enhancing phosphate uptake under the condition of Pi starvation, and in the translocation of Pi among cells in shoots to increase the efficiency of internal Pi use. Similar to *OsLPTs*, in situ hybridization and RT-PCR analysis demonstrated that the expression of *OsPT6:1*, another rice Pi transporter gene, is also expressed in both roots and leaves, with the peak expression signal observable in mesophyll cells under low phosphorus (P) induction (Ming et al., 2006). The tomato Pi transporter gene *LePT1*, with the highest concentrations in root hairs, is demonstrated to be cell-specific expressed. The *LePT1* mRNA is detectable in peripheral cell layers such as rhizodermal and root cap cells. The mRNA levels are also detectable in young stelar tissue under Pi-deprivation condition (Daram et al., 1998). Taken together, the pattern of response to Pi and distinct organ or tissue expression model of Pi transporter genes present molecular and biochemical evidence that the plant Pi transporter genes are transcriptional regulated via various molecular pathways, which further execute the functions of Pi acquisition at the root/soil interface and Pi translocation within the cells in plants.

4.2 Expression pattern in response to Pi

Lots of studies have revealed a marked increase in the transcripts of Pi transporter genes under Pi starvation. The genes encoded high-affinity Pi transporters are generally up-regulated, showing that the transcript levels of high-affinity Pi transporter genes increased rapidly as a specific response to Pi starvation (Muchhal et al., 1996; Liu et al., 1998a). Currently, the expression of Pi transporter genes significantly up-regulated under Pi deprivation have been demonstrated in various plant species, such as *Arabidopsis AtPT1* and *AtPT2* (Karthikeyan et al., 2002) and *pht1;4* (Misson et al., 2004), rice *OsPT1* (Seo et al., 2008), *OsPT2* and *OsPT6* (Ai et al., 2008), potato *StPT1* and *StPT2* (Leggewie et al., 1997), *Dunaliella viridis DvSPT1* (Li et al., 2006), wheat *TaPHT1.2* (Miao et al., 2009) and *TaPT2* (Tittarelli et al., 2007), *M. truncatula MtPT1* and *MtPT2* (Liu et al., 1998b), barley *HORvu*;*Pht1;1* (Rae et al., 2003), maize five encoding Pht1 phosphate transporter genes (Nagy et al., 2006), tomato *LePT1* (Muchhal et al., 1999; Liu et al., 1998a) and *LePT2* (Liu et al., 1998a). These results provide the evidence that plant high-affinity Pi transporter genes are generally transcriptional activated

during Pi deficiency (Muchhal et al., 1999). These results also suggest that Pi transporter genes play important roles in phosphate absorption and translocation from the growth medium to the different parts of the plant (Misson et al., 2004).

4.3 Expression pattern in response to other nutrients

The plant Pi transporter genes are mainly regulated by Pi. The low-Pi induced tomato *LePT1* and *LePT2* are not regulated by starvation of nitrogen, potassium, or iron (Liu et al., 1998a). In the potato *StPT2*, with the transcripts mainly detected in root organs when plants were deprived of Pi, no expression was found when other macronutrients were lacking (Leggewie et al., 1997). These results suggest that the plant Pi transporter genes are generally to be specifically responsive to Pi. However, the expression patterns of some Pi transporters can also be regulated by other inorganic nutrients. The barley high-affinity Pi transporter genes *HVPT1* and *HVPT2*, are intimately linked to the zinc (Zn) status of the plants besides their response to Pi-starvation. Zn deficiency induced the expression of the two genes in plants grown in either P-sufficient or-deficient conditions. Moreover, the role of Zn in the regulation of these genes is specific and cannot be replaced by manganese, a divalent cation similar to Zn (Huang et al., 2000). This result suggests that Zn plays a specific role in the signal transduction pathway responsible for the regulation of genes encoding some high-affinity P transporters in plant roots. The significance of Zn and other inorganic nutrients involvement in the regulation of Pi transporter genes and the corresponding mechanism need to be classified in the future.

4.4 Expression pattern in response to environmental factors

Several other factors except the status of Pi and other inorganic nutrients, such as Zn, have also endowed their effects on the expression of part Pi transporter genes. It is found that the *Pht2;1* is strongly up-regulated by light in potato and *Arabidopsis* leaf tissue (Rausch et al., 2004). *DvSPT1*, a *Dunaliella viridis* sodium-dependent phosphate transporter gene, was induced upon NaCl (Li et al., 2006). The birch *Mpt1* transcript levels are significantly up-regulated by ozone oxidative stress in a short time (2 h) (Kiiskinen et al., 1997). In petunia corollas, a high-affinity phosphate transporter gene *PhPT1* (*PhPht1;1*), which shows to be up-regulated during senescence and possibly to be functional in Pi translocation during senescence, shows a rapid increase followed with low levels of ethylene treatment (0.1 mM) (Chapin and Jones, 2009). The mRNA accumulation of *PsMPT* in tree peony was strongly induced by chilling treatment during the release of bud dormancy (Huang et al., 2008). Together, these results imply that the plant Pi transporter genes have a complicated transcriptional mechanism regulated by many environmental factors, which include Pi, Zn and other unknown inorganic nutrients, and other factors.

5 Regulated expression of plant Pi transporter genes via arbuscular mycorrhizal symbiosis

Arbuscular mycorrhizal (AM) symbiosis is a mutualistic endosymbiosis formed by plant roots and AM fungi. For a long time, it has been clearly known that most vascular flowering plants have the ability to form these associations, which have a significant impact on plant health and consequently on ecosystem function (Javot et al., 2007). One central feature of the AM symbiosis is the nutrient exchange in which AM fungi obtain carbon from their plant host while assisting the plant with the acquisition of phosphorus (as phosphate) from the soil (Karandashov and Bucher, 2005; Javot et al., 2007).

In arbuscular mycorrhizas, fungal hyphae often extend between the root cells and tuft-like branched structures (arbuscules) form within the cell lumina that act as the functional interface for nutrient exchange (Rausch et al., 2001). Pi transporters, the important components of Pi transferring under AM symbiosis, display a distinct expression pattern. Currently, it is known that mycorrhiza-specific phosphate transporter genes and their regulation are conserved in phylogenetically distant plant species, and they are activated selectively by fungal species from the phylum Glomeromycota (Karandashov et al., 2004).

So far, lots of Pi transporter genes within plants with variable expression levels have been demonstrated under AM symbiosis. In legume model plant *Meticago truncatula*, among the six genes from the Pi transporter gene family, *MtPT4* was induced and other five genes showed different degrees of repression by three fungi including *Grammostola rosea*, *Glomus mosseae* and *Glomus intraradices* (Grunwald et al., 2009). Another study on *MtPT4* also explores that this gene is expressed only in the AM symbiosis, being essential for the acquisition of P(i) delivered by the AM fungus (Javot et al., 2007). In tomato, three phosphate transporters (*LePT3*, *LePT4*, and *LePT5*) are up-regulated upon colonization by arbuscular mycorrhizal fungi. Mycorrhizal infection at a Cp of 0.05 mM resulted in a significant increase in the transcripts of *LePT4* in the wild type and a concomitant 2-fold increase in Pi uptake (Nagy et al., 2005; Xu et al., 2007).

Under AM symbiosis condition, the locations to exchange the Pi between the symbiotic partners via plant or fungi Pi phosphate transporters have been paid more attention to. The potato phosphate transporter gene, *StPT3*, is expressed in a temporally defined manner in root cells harboring various mycorrhizal structures, including thick-coiled hyphae (Karandashov et al., 2004). Located in the membrane fraction of mycorrhizal roots, MtPT4 is

colocalized with the arbuscules, consistent with a location on the periarbuscular membrane. The transport properties and spatial expression patterns of *MtPT4* are consistent with a role in the acquisition of phosphate (Harrison et al., 2002). The results highlight the role of different symbiotic structures in phosphorus transfer, and they indicate that cell-cell contact between the symbiotic partners is required to induce phosphate transport.

Not only part of plant Pi transporter genes are induced upon the AM fungi symbiosis, distinct members of Pi transporter genes in the *Arbuscular mycorrhizal* (AM) fungi are also up-regulated after the fungi infection. Harrison et al. (1995) have identified a cDNA that encodes a transmembrane phosphate transporter (GvPT) from *Glomus versiforme*, a VA mycorrhizal fungus. Expression of *GvPT* was localized to the external hyphae of *G. versiforme* during mycorrhizal associations, demonstrating these being the initial site of phosphate uptake from the soil (Harrison and van Buuren, 1995). The two Pi transporter genes in fungi *H. cylindrosporum*, *HcPT1* and *HcPT2*, are induced after associating with its natural host plant, *Pinus pinaster*, under either low or high P conditions. The up-regulation of *HcPT1* and *HcPT2* further increases net Pi uptake rates into intact *Pinus pinaster* roots at low or high soil P levels (Tatry et al., 2009). So far, though the identified genes encoding mycorrhiza-specific plant phosphate transporters have enabled fundamental problems in Arbuscular mycorrhizal symbiosis research to be partly elucidated, the study of phosphate transport mechanisms and their gene regulation will be further stressed based on which we can enlarge our understanding of the intimate interaction between the two symbiotic partners (Karandashov and Bucher, 2005).

6 Transcription regulation of plant Pi transporter genes

Most of the Pi transporter genes are shown to be responsive to environmental Pi. Thus, it is valuable to explore the molecular mechanism of the transcriptional regulation in plant Pi transporter genes. An effective approach to study transcriptional regulation is to monitor the activity of reporter genes driven by the specific gene promoter. Expression of reporter genes such as luciferase (LUC), green fluorescent protein (GFP), and β-glucuronidase (GUS) has been successfully used to understand transcriptional regulation in plants (Ow et al., 1986; Jefferson et al., 1987; Haseloff et al., 1997).

So far, important promoter regions and regulatory elements controlling gene regulation specificities have been identified in plant Pi transporters. In *Arabidopsis*, the transgenic plants fused the expression cassettes with truncated promoter fragments of *AtPht1;4* cloned to GUS coding sequence were developed. The results indicate that that expression of *AtPht1;4* in the roots and aerial parts is regulated by independent mechanisms (Karthikeyan et al., 2009).

Adopting the promoter deletion analysis approach, it is found that one PHO-like motif and three motifs similar to the dicot P1BS element identified in barley HvPht1;1 promoter play important roles in the regulation of the response to Pi in the Pi transporter gene. Mutation of the P1BS elements eliminated any induction of gene expression in response to low Pi. An internal HvPht1;1 promoter fragment, incorporating a single P1BS element, had an increased response to P deprivation in comparison with the unmodified promoter (Schünmann et al., 2004b). Furthermore, the promoters of other six barley Pi transporter genes have also been detected for the presence of regulatory elements (Schünmann et al., 2004). These findings provide direct evidence for a functional role of the P1BS element in the expression of P-regulated genes. In wheat, promoter deletion analysis finds that a 579 bp fragment of the TaPT2 promoter is sufficient to drive the expression of the *GUS* reporter gene specifically in roots of Pi-deprived plants (Tittarelli et al., 2007). The regulatory element in Pi response, P1BS, is also identified in TaPHT1.2-D, another wheat Pi transporter (Miao et al., 2009). Further comparison analysis suggested that conserved regions and candidate regulatory motifs similar to those in barley, rice, and *Arabidopsis* were detected, indicating that there are conserved cis-acting elements and trans-acting factors that enable the TaPT2 promoter to be regulated in a tissue-specific and Pi-dependent fashion in both monocots and dicots (Tittarelli et al., 2007).

The responses to Pi status in the Pi-responsive genes are determined by the regulatory elements which locate at the promoter region and the distinct transcription factors that interact with the elements. The *Arabidopsis* PHO1;H1, being involved in the Pi homeostasis within plants and being low-Pi responsive, is regulated by a MYB type transcription factor PHR1 (Stefanovic et al., 2007). However, very few transcription factors that interact with the putative regulatory elements P1BS and PHO-like motif have been identified. Therefore, the upstream factors that function on the transcriptional regulation of Pi transporter and the distinct signal transduction pathways involved in response to Pi in various Pi transporter genes are largely unknown and need to be further studied.

7 Functional analysis of plant Pi transporter genes

7.1 Functional identification based on genetic complement technique

Because the Pi transporter genes within plants exist as a large gene family which may cause overlap or redundancy in the gene function, it is not effective to identify the functions of a single Pi transporter in plant systems

(Hürlimann et al., 2009). Therefore, a single cell system with the mutation of Pi acquisition has been widely used for the analysis of function and corresponding biochemical properties of plant Pi transporters. For some time, the mutant yeast cells with drawback of Pi acquisition has been demonstrated as an effective host for the identification of the heterogonous Pi transporter functions. Functions of various Pi transporters derived from different plant species have been evaluated in the mutant yeast expression system, including tobacco *NtPT1* (Baek et al., 2001), high-affinity *Arabidopsis* AtPT1 and AtPT2 (Muchhal et al., 1996), Pht2;1 (Daram et al., 1999), PHT2;1 (Versaw et al., 2002), potato StPT1 and StPT2 (Leggewie et al., 1997), and StPT3 (Rausch et al., 2001), wheat TaPT2 (Zeng et al., 2002), rice OsPT11 (Paszkowski et al., 2002), MtPT1 (Liu et al., 1998b), MtPT4 (Javot et al., 2007), and tomato LePT4 (Xu et al., 2007). The phenotype of yeast strain mutated with Pi acquisition capacity are generally adopted to be a high affinity phosphate transporter (pho84) mutant strain MB192 (Paszkowski et al., 2002; Zeng et al., 2002) and NS219 (Leggewie et al., 1997). Other than the mutant yeast cells, the Xenopus oocytes have also been successfully used to identify the functions and the biochemical properties of several Pi transporters in plant species. By injection of *OsPT2* mRNA into the cells of Xenopus oocytes, a Pi-elicited depolarization of the cell membrane electrical potential could be established when supplied with mM external concentrations, concurrently with an increased Pi accumulation. The results show that OsPT2 mediated the uptake of Pi in oocytes (Ai et al., 2008).

7.2 Functional analysis of plant Pi transporter genes based on mutants and transgenic analysis

In the past several years, some mutations of Pi transporter genes identified in plant species have provided a novel way to explore some native functions of the plant Pi transporters. Based on *Arabidopsis* mutation stock screening and mutated target gene identification, a mutant of Pht1;4, a member of the Pht1 family, has been isolated. It is found that the Pi content in the mutant was not significantly different from that of the corresponding wild type controls. Furthermore, the mutant did not display any obvious growth defects or visible phenotypes when grown on a low phosphate containing medium (Misson et al., 2004). This study offers a first step in the complex genetic dissection of the phosphate transport system in plants. To elucidate the roles of Pht1;1 and Pht1;4 in plants, Shin et al. (2004) created a double mutant that lacked both *Pht1;1* and *Pht1;4* based on the identification of mutants with a single gene of loss-of-function and the further cross of the two single mutants. Consistent with their spatial expression patterns, membrane location and designation as high-affinity Pi transporters, Pht1;1 and Pht1;4 contribute to Pi transport in roots during growth under low-Pi conditions. The double mutant also shows a 75% reduction in Pi uptake capacity during growth under high-Pi conditions relative to wild type (Shin et al., 2004). This study suggests that the mutated Pi transporter genes and their further combination provide an effective way for the dissection of the functions of the members in the Pi transporter gene family within the plants.

Genetic transformation of plant Pi transporter genes for evaluation of the Pi transporter functions have been conducted by several research groups. By transforming the suspension cells of an *Arabidopsis* Pi transporter gene *ANTR1*, the Pi uptake property regulated by ANTR1 has been analyzed in the transformed cells (Pavón et al., 2008). In rice, the knock-down of either *OsPT2* or *OsPT6* expression by RNA interference significantly decreased both the uptake and the long-distance transport of Pi from roots to shoots. The results also suggest OsPT6 plays a broad role in Pi uptake and translocation throughout the plant, whereas OsPT2 is mainly involved in translocation of the stored Pi in the plants (Ai et al., 2008). For determination of the biochemical properties of barley HORvu;Pht1;1 and HORvu;Pht1;6, the genes were over-expressed in rice plants under the control of the rice actin promoter and suspension cell cultures were generated. It is observed that the cells derived from transgenic plants were able to take up phosphate at a much higher rate than control cells. The Km values for phosphate in cells expressing HORvu;Pht1;1 and HORvu;Pht1;6 and the corresponding biochemical properties in relation to the Pi transportation have been explored (Rae et al., 2003). Therefore, the genetic transformation of Pi transporter genes in plants could be used as a supplemental pathway to dissect the functions of plant Pi transporter genes.

7.3 Application perspectives of potential plant Pi transporter genes via gene engineering

Genetic engineering has become a potential approach to improve the phosphate use efficiency in plants. Such approaches should assist in the breeding of crop plants that exhibit improved P acquisition efficiency and thus require lower inputs of P fertilizer for optimal growth (Bucher et al., 2007). In the past decade, with the information on the cell types in which phosphate transporters are expressed, together with functional characterization of the transporters being available, the functional Pi transporter genes have provided valuable gene resources in the improvement of phosphorus nutrition in plants (Smith et al., 2000).

In rice, it is observed that the transgenic rice plants overexpressing the *OsPT1* gene accumulated almost twice as much phosphate in the shoots as did wild-type plants. As a result, transgenic plants had more tillers than did wild-type plants, which is a typical physiologic indicator for phosphate status in rice (Seo et al., 2008). High expression of the *Arabidopsis* gene *PHT1*, a higher plant homolog to the high-affinity phosphate transporter gene of yeast (*Saccharomyces cerevisiae*) PHO84, can obviously

increase the rate of phosphate uptake in tobacco-cultured cells. The transgenic cells also exhibit increased biomass production when the supply of phosphate was limited, suggesting that establishment of gene engineering of phosphate transport could be used as a powerful way toward enhancing plant phosphorus nutrition (Mitsukawa et al., 1997). The plant Pi transporter genes with potential significantly increased capacity of Pi uptake under deficient-Pi condition provide a new pathway for the improvement of plant phosphorus nutrition based on genetic transformation approach in the future.

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